Supplemental Methods

Western blot antibodies and procedure

The following primary antibodies were used: HIF-1α and HIF-2α antibodies (Novus Biologicals), VHL antibody (BD Biosciences Pharmingen), actin antibody (ICN Biomedicals), Jagged-1 antibody (Santa Cruz Biotechnology), Notch-1 antibody (Santa Cruz Biotechnology), Hes-1 antibody (kindly provided by Dr. Tetsuo Sudo), $p21^{Cip1}$ antibody (DAKO) and $p27^{Kip1}$ antibody (DAKO). The Notch-2 (C651.6DbHN) antibody developed by Dr Spyros Artavanis-Tsakonas was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA, USA. HRP-conjugated secondary antibodies were obtained from Amersham Biosciences and Jackson ImmunoResearch Laboratories Inc.. Super Signal substrate (Pierce) was used for chemiluminescence detection.

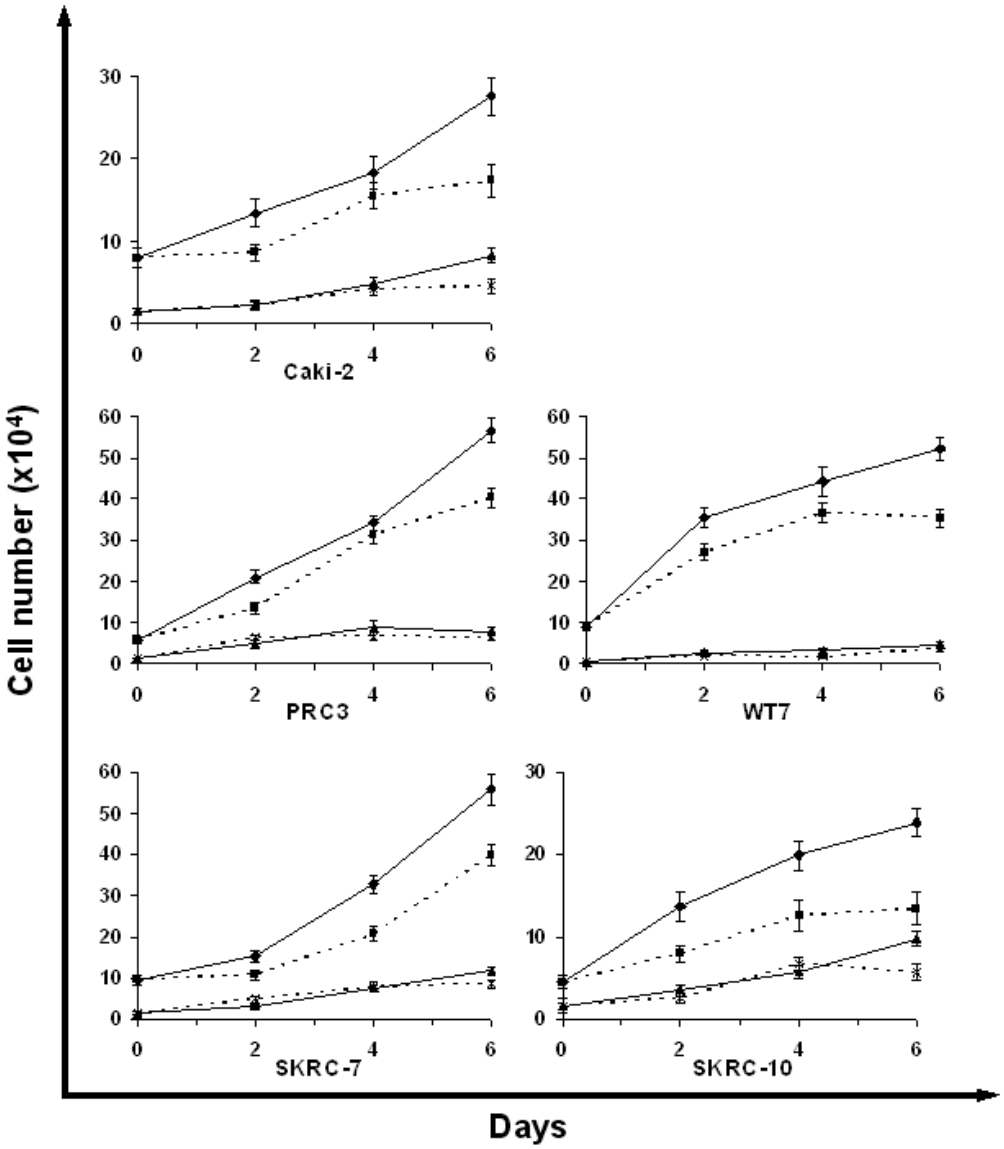
Supplemental Table

Supplemental Table I. Sequences of primers used in Q-PCR reactions.

Gene	Forward (5'-3')	Reverse (5'-3')
Jagged-1	CAACACGGTCCCCATCAAG	TACTTCAGAATTGTGTGTCCTTATTTTAGA
Jagged-2	GGCACTCGCTGT ATGAAAGGA	GCACAACCTCTGGTAACAAACG
Dll-1	GGTCATGGAGTTGTCATTCGTCTA	TATCATTTCCTGTGCCAACTCTTTT
Dll-3	CCCTACCCTTCCTCGATTCTG	GAACTGAAAATGGGCTTAAAACCTT
Notch-1	CCGCAGTTGTGCTCCTGAA	ACCTTGGCGGTCTCGTAGCT
Notch-2	GGCATTAATCGCTACAGTTGTGTCT	GGAGGCACACTCATCAATGTCA
Notch-3	TGATCGGCTCGGTAGTAATGC	GACAACGCTCCCAGGTAGTCA
Hes-1	AGCGGGCGCAGATGAC	CGTTCATGCACTCGCTGAA
Hey-1	CTTGAGTTCGGCTCTGTGTTCC	GATGCCTCTCCGTCTTTTCCT
Hey-2	TCGCCTCTCCACAACTTCAGA	GAATCCGCATGGGCAAAC
HIF-1α	TTCCAGTTACGTTCCTTCGATCA	TTTGAGGACTTGCGCTTTCA
VEGF	AGGAGGAGGGCAGAATCATCA	CTCGATTGGATGGCAGTAGCT
CAIX	CCAGGCCTCACTGGCAACT	TCGCCCAGTGGGTCATCT

Supplemental Figures

Supplemental Figure 1. Inhibition of Notch signaling impairs growth of CCRCC cells. The number of viable (♦ = DMSO and ■ = DAPT) and dead cells (▲ = DMSO, TB positive (+) and x = DAPT, TB positive) was determined by TB exclusion at indicated times in a panel of CCRCC cells treated with DMSO or DAPT. Results expressed as mean ± SEM of one representative experiment performed in triplicates.



Supplemental Figure 2. γ-secretase inhibition does not affect mouse weight. Nude mice were treated in cycles of three days (⟷) with daily injections with DAPT (10 mg/kg/day) or vehicle control followed by four days without treatment. Data represent the mean mouse weight (g) + SEM of DAPT (n=6) or vehicle (n=10) treated mice.